

**Assessing Beaked Whale Reproduction and Stress  
Response Relative to Sonar Activity at the Atlantic Undersea  
Test and Evaluation Center (AUTECH)**

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## **LONG-TERM GOALS**

Atypical mass strandings and behavioral responses of beaked whales have been correlated with exposure to naval sonar (e.g. Simmonds and Lopez-Juraco 1991; Frantz 1998; Evans and England 2001), highlighting a need to understand the potential physiological impacts to individual whales and if these in turn represent a biologically significant threat to exposed populations. The long-term goal of this study is to assess glucocorticoid levels from blubber biopsies of targeted species, to assess stress levels relative to sonar exposure. Specifically, the project aims to collect biopsy samples at the U.S. Navy's Atlantic Undersea Test and Evaluation Center (AUTECH) in the Andros-AUTECH Operating Area where fleet readiness training involves regular use of mid-frequency active sonars, and compare the levels to those measured in biopsies collected from control populations within the Bahamas region that are less exposed to sonar activity. In parallel, pregnancy states will be ascertained via blubber progesterone levels in both groups of animals to investigate whether there is a relationship between sonar activity, stress measures, and reproductive rates, to assess population-level impacts.

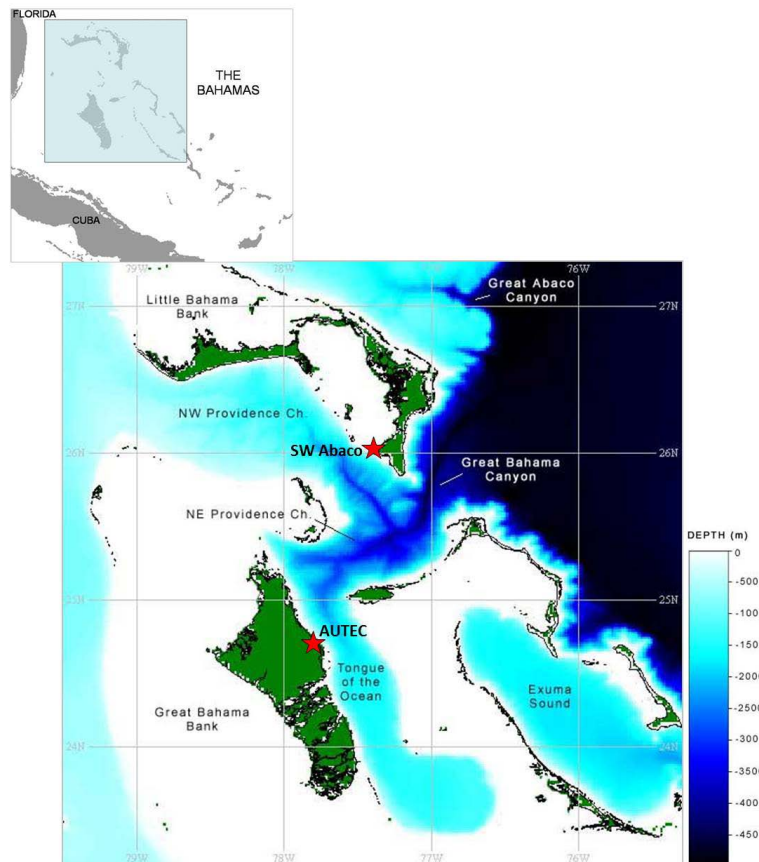
## OBJECTIVES

The primary objectives of the study are:

- 1) To assess stress levels measured from glucocorticoid concentrations in blubber biopsies relative to sonar activity, and relate these to pregnancy rates from progesterone concentrations in the same tissue for Blainville's beaked whales (*Mesoplodon densirostris*) and sperm whales (*Physeter macrocephalus*).
- 2) To collect photo-identification data to monitor repeated sampling of individuals, construct sighting histories and identify consistent associates as covariates for stress analyses; and to document successful calving events for comparison to hormone-derived pregnancy rates.

## APPROACH

The experimental design of this study is based on a population comparison: to compare beaked whale and sperm whale stress levels and pregnancy rates between areas with contrasting sonar activity: at the U.S. Navy's Andros-AUTEC Operating Areas in Tongue of the Ocean (TOTO) and off the southwest coast of Abaco Island and other regions throughout the Great Bahama Canyon but outside of TOTO (Figure 1).



**Figure 1. Map of the northern Bahamas showing the location of the two areas where blubber biopsy samples were primarily collected: AUTEC and SW Abaco. Both lie within the Great Bahama Canyon, a submarine canyon that reaches depths of more than 4000m. Additional sampling occurred throughout the canyon region.**

Stress levels for whales at AUTECH are being evaluated using biopsy samples collected primarily before scheduled Submarine Commanders Courses (SCC) as part of a Living Marine Resources (LMR) funded satellite tagging study. Five 15-day field efforts were planned under this study, in Oct/Nov 2011 and 2013, July 2012 and April/May 2011-14. Reproductive rates are being examined relative to measured stress levels and sonar activity on the range during the preceding months through collaboration with David Moretti at the Naval Undersea Warfare Center. A further 15-day summer sampling effort was conducted at AUTECH in summer 2012 and 2013. In addition, three 15-day field efforts have been conducted in the control area at SW Abaco, spanning May 2011- May 2013, closely matching the timing of the spring SCC at AUTECH. These control samples are being augmented by samples collected during a concurrent annual SERDP-funded sightings-survey around the northern Bahamas, as well as samples collected opportunistically as part of the ONR-funded Bahamas Beaked whale Ecology Study (N000140710120) until that project ended in May 2012. The species targeted are Blainville's beaked whale (*Mesoplodon densirostris*) and sperm whale (*Physeter macrocephalus*).

Using AUTECH's instrumented array of bottom-mounted hydrophones on the Weapons Range, beaked whales and other odontocetes can be monitored and localized in real time by passive acoustic detection of their echolocation clicks (DiMarzio *et al.* 2008). Acoustic technicians from the Naval Undersea Warfare Center relay real-time cetacean localizations using the marine mammal monitoring system at AUTECH and direct observers on a 6.8 m rigid-hulled inflatable (RHIB) to the whales, increasing the opportunities for locating animals and obtaining biopsies. No such array exists off SW Abaco Island; so instead, boat-based surveys are concentrated in areas of known higher density of beaked whales, identified from over a decade of research in the area (Claridge 2006). A hand-held hydrophone is deployed to detect foraging sperm whales. Combined these approaches increase opportunities for finding animals off Abaco Island.

When animals are located, remote biopsy sampling (e.g., Hooker *et al.* 2001) is being used to obtain skin and blubber biopsies. Stress levels are being examined relative to sonar activity by measuring glucocorticoid concentrations in blubber biopsies (Kellar *et al.* 2015). Pregnancy state will also be assessed by measuring progesterone concentrations in blubber (Kellar *et al.* 2006; Trego *et al.* 2013), and pregnancy rates will be examined relative to measured stress levels by comparison between samples collected at AUTECH and the control area around Abaco.

Photo-identification data are providing a record of all individuals sighted, and being used to build sighting histories, using new data and the existing BMMRO database. These data will provide information on ranging patterns and demographics that can serve as covariates for analysis of stress patterns. Similarly, photo-identification data will be used to evaluate the stability of individual associations to identify consistent associates that may have similar exposure and stress levels. Longitudinal photo-identification records will also enable documentation of successful calving events, to compare with hormone-derived pregnancy rates.

## **WORK COMPLETED**

The field work component of this project was completed in 2014. When blubber samples collected during field work conducted for this study from FY11-14 are tallied, we have a total of 42 Blainville's beaked whale samples (10 from AUTECH and 32 from SW Abaco) and 32 sperm whale samples (17 from AUTECH and 15 from SW Abaco) for use in the hormone analyses (Table 1). These will be augmented by 41 additional Blainville's beaked whale and 12 Gervais' beaked whale samples

remaining from previous work as well as samples currently being processed for the SERDP project, some of which have been made available.

**Table 1. Summary of blubber biopsies collected for target species at AUTEK and SW Abaco from FY11-FY14 during this study. These were augmented by additional samples collected during previous work within and outside of Tongue of the Ocean (TOTO).**

Year	Blainville's beaked whale		Sperm whale	
	AUTEK	SW ABACO	AUTEK	SW ABACO
FY11	0	5	8	2
FY12	4	24	4	12
FY13	6	3	3	1
FY14	0	0	2	0
<b>Total</b>	<b>10</b>	<b>32</b>	<b>17</b>	<b>15</b>

Of the 83 Blainville's beaked whale samples, 81 (15 AUTEK and 66 Abaco) have been processed for blubber cortisol, progesterone, and percent lipid. The remaining two samples contained insufficient blubber for processing. Of the 32 sperm whales samples, 21 have been processed for blubber cortisol, progesterone, and percent lipid. The remaining sperm whale samples are currently being processed with a completion date estimated to be 30 November 2015. Sexes of sampled animals were determined using genetic assays and pregnancy determination was made using 10ng of progesterone/g of blubber as the threshold between pregnant and non-pregnant females. This threshold is lower than that used for other cetaceans, typically closer to 20-30ng/g, but the entire distribution of pregnant *Mesoplodon* specimens analyzed appeared to be substantially lower than that for other cetaceans. There was also a non-continuous lapse (i.e., break) observed in the sequential distribution of progesterone values between ~5 ng/g and 10 ng/g with the highest male at 2.49ng/g. Without measurements from additional animals of known reproductive state (especially pregnant females) this threshold is set somewhat arbitrary but judged and informed by the authors' experience with over 2000 processed samples representing 18 cetacean species. Future work will include determining the reproductive state of individuals when sampled using the photo-identification data when available to further assess this chosen threshold.

Two types of statistical approaches were used to analyze the data. Bivariate comparisons between the different species were conducted on each of the five metrics analyzed: cortisol concentration, progesterone concentration, proportion female, proportion pregnant, and lipid composition using delta mean permutation tests (akin to a t-test with fewer implied assumptions). In addition, within each species, blubber cortisol was modeled as a function of the following covariates: area, sex, pregnancy state, location (expressed as latitude given the north/south distribution of the samples), time (expressed as ordinal date from the year of initial sampling), Julian date (i.e., season, irrespective of year) and %lipid by mass of the biopsy tissue. Covariate associations with blubber cortisol were assessed as part of a Bayesian model averaging procedure (with a generalized linear model framework) using a method in which the model estimation process also selects which factors to include and which to exclude for each iteration of a Markov chain Monte Carlo (MCMC) run (Carlin and Chib 1995). In this analysis, sets of logistic generalized linear models were constructed in WinBUGS.

## RESULTS

**Note:** The following results reflect a rapid initial analysis of the hormone and lipid data and should be viewed as preliminary. In this preliminary report, reference to “AUTECH” includes all samples collected throughout Tongue of the Ocean and “Abaco” refers to all samples collected outside TOTO, and not just SW Abaco.

### *Interspecies analysis*

There were large differences between the sperm whales and the two *Mesoplodon* species across all metrics evaluated. As such species was the dominate factor associated with the majority of variation in blubber cortisol concentration, blubber progesterone concentration, percent lipid of the blubber, sex composition of the sample group, and proportion of females that were pregnant. Sperm whales had significantly lower cortisol, progesterone (largely because there were no pregnant animals sampled), and %lipid values compared to their *Mesoplodon* counterparts (Table 2). In addition, there were no identified pregnancies in the 8 females sperm whales sampled compared with more than 1/3 of the sampled female Blainville’s beaked whale were identified as pregnant (18/49).

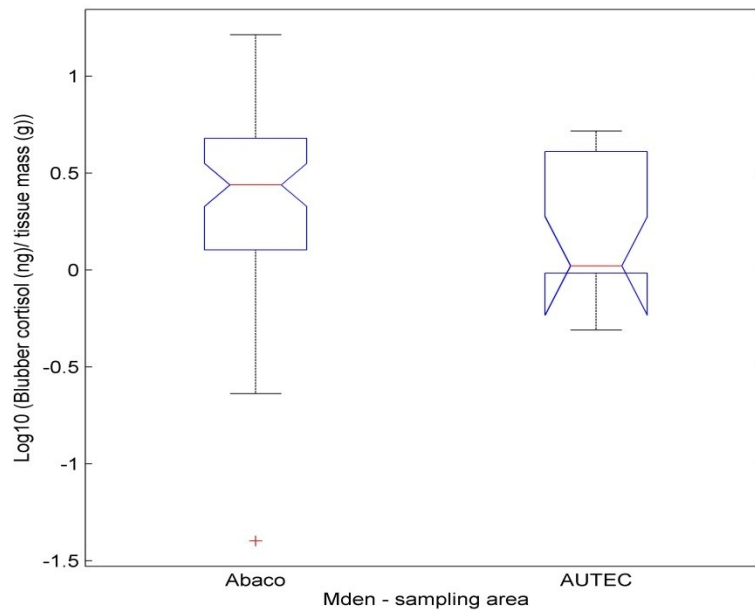
**Table 2. Summary of mean blubber hormone concentrations, the fraction of females collected in the sample and the fraction of those females that were pregnant. Mden = Blainville’s beaked whale, Pmac = sperm whale, Meur = Gervais’ beaked whale.**

Species-pop (number of samples)	Mean blubber hormone(ng)/tissue(g)		Demography		Fat composition  % lipid (SE)
	Cortisol (SE)	Progesterone (SE)	Female/Total	Pregnant /Female	
Mden-other (66)	3.36 (0.36)	10.98 (3.1)	40/66	15/40	31.3% (1.3%)
Mden-AUTECH (15)	2.23 (0.48)	10.81 (6.1)	9/15	3/9	37.7% (2.5%)
Pmac-other (12)	0.91 (0.28)	2.47 (0.6)	8/12	0/8	6.5% (1.7%)
Pmac-AUTECH (9)	1.42 (0.71)	0.99 (0.1)	0/9	0/0	7.8% (2.0%)
Meur-Abaco (12)	6.55 (2.70)	2.98 (1.2)	3/12	2/3	30.7% (2.5%)

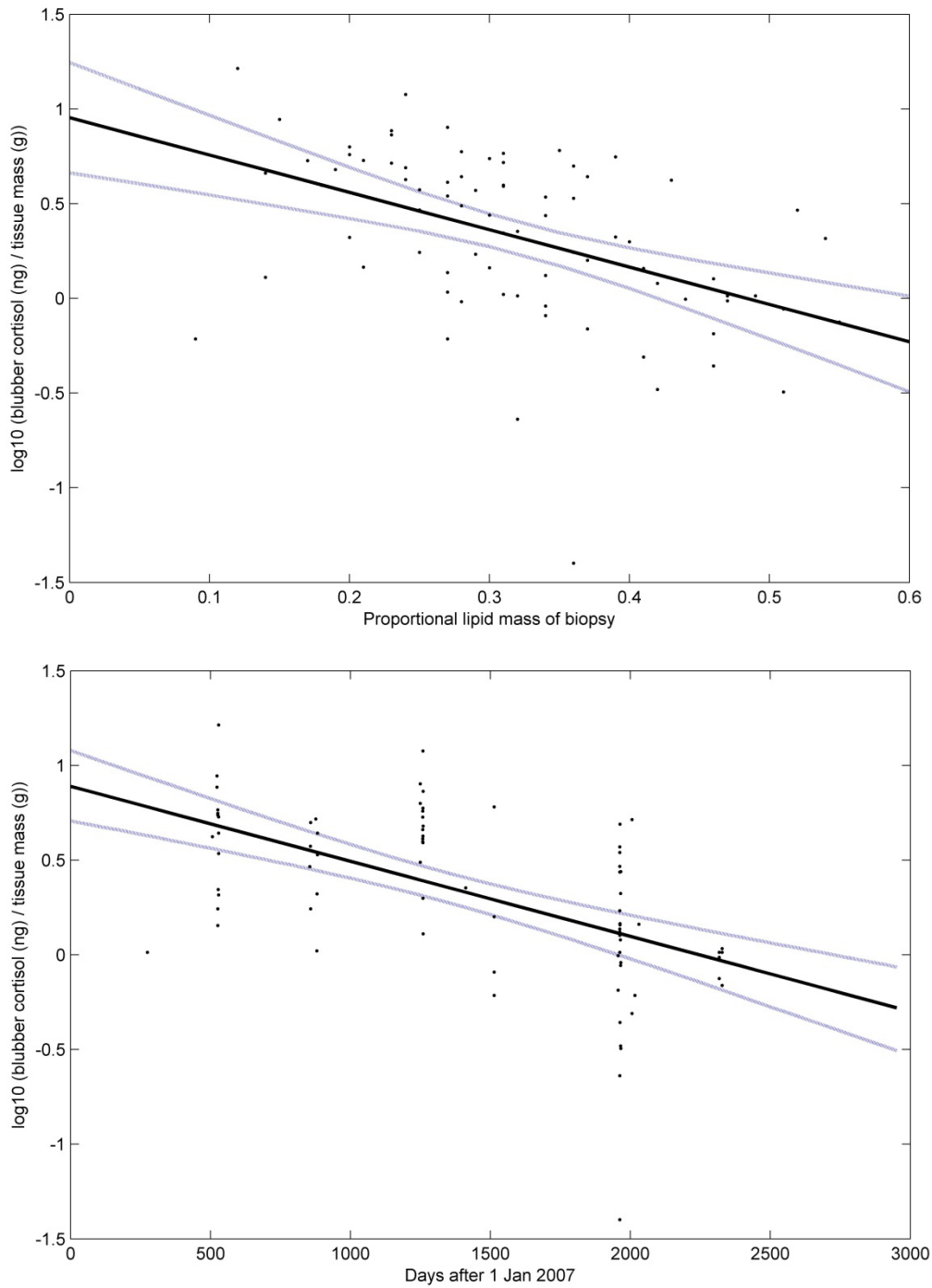
### *Blainville’s beaked whale analysis*

The intra-species comparisons were much less distinct. The Abaco (outside TOTO) and AUTECH (TOTO) Blainville’s beaked whales showed no significant differences between each other in any of the metrics measured: cortisol concentration, progesterone concentration, proportion female, proportion pregnant, or lipid composition (Figure 2). A Bayesian model selection technique indicated the weight of evidence favored a negative relationship between cortisol of Blainville’s beaked whales and (1) the % lipid of the sampled blubber (Figure 3a) and (2) year or ordinal date of the sampling (Figure 3b) (see also Appendix Table 1a). In other words there were higher measured cortisol values during a period in which the samples collected had low % lipid (2008-2010) and lower cortisol concentrations during the

period in which samples exhibited higher % lipid values (2011-2013). These analyses will be rerun comparing only whales that were sampled at AUTEC and SW Abaco.



**Figure 2. Box plot characterizing the distributions of blubber cortisol values in Abaco (outside TOTO) and AUTEC (TOTO) Blainville's beaked whales (noted here as Mden). The weight of evidence indicated that there was no to weak differentiation between the areas' cortisol values. Horizontal box lines represent the lower quartile, median and upper quartile values. Whiskers lines indicate range of concentrations. Points of inflection represent upper and lower bounds to the 95% confidence interval.**



**Figure 3. Scatter plots showing the relationship in Blainville's beaked whales between  $\log_{10}$  (blubber cortisol) and %lipid (proportion of the mass of the sample biopsy that was lipid) (A) and day of sample (number days after Jan1 2007) (B). There was strong evidence of a statistical relationship between these two cofactors and blubber cortisol concentration. This A was used merely as a reference point. The lighter lines represent bootstrapped 95% confidence envelopes on the best-fit outcomes of the linear relationships (dark lines).**

### *Sperm whale analysis*

The sperm whales sampled off Abaco (outside TOTO) and AUTECH (TOTO) show no significant differences with most of the covariates evaluated. The sole exception was sex composition with disproportionately more females sampled off Abaco. The Bayes model selection yielded only Julian date (i.e., season, irrespective of year) as a covariate of importance in blubber cortisol variation (see Appendix 1 Table 1b).

### *Gervais' beaked whale analysis*

There were no Gervais' beaked whales sampled at AUTECH (TOTO) and therefore no data for area comparisons. Moreover, the weight of evidence from the model selection technique for was the exclusion of all the covariates from the model, i.e., none were important in their association with blubber cortisol concentration (see Appendix 1, Table 1c).

### *Preliminary conclusions*

The preliminary analysis shows no difference in Blainville's beaked whale mean blubber cortisol concentrations or pregnancy rates between the sonar exposure area (TOTO) and our reference area (outside TOTO). The next important analysis stages will be three fold. The first will be to examine the reproductive success rate of photographed pregnant females to assess the relative likelihoods of producing viable calves (i.e., one that survives for sufficient time to be observed and photographed). This has proven to be an important metric of population health in animals exposed in oil spills (unpublished data, Kellar). The second will be to conduct a more fine-scale analysis of the areas examined during this study. For this preliminary analysis, all animals in the Tongue of the Ocean were grouped as "AUTECH" individuals, though some were sampled off the range itself, while all whales sampled outside of TOTO were grouped as "Abaco" when in fact some of these were sampled in nearby NE Providence Channel, much closer to AUTECH than to SW Abaco. As such these differences will be re-examined for the final report. The third will be to conduct additional analysis with respect to the temporal sonar usage on AUTECH. Given the small sample set from this area additional sampling is recommended.

The relationship between blubber cortisol and %lipid composition in sampled Blainville's beaked whales is an indication that either animals that are experiencing greater stress response are less successful when foraging or more likely that individuals that are nutritionally stressed produce more cortisol to mobilize energy from existing stores (Kellar et al. 2015). Additional analysis is required to further elucidate this relationship however it is promising for this technique, i.e., using biopsies for population health assessments.

## **IMPACT/APPLICATIONS**

Improving our understanding of the population responses of beaked whales relative to sonar usage will aid the US Navy in assessing the potential need for additional mitigation practices for protected marine mammals. In particular, central questions for effective management and potential mitigation are whether sonar use causes detectable physiological stress responses and whether these responses are linked to biologically significant reductions in population health or condition.

This study continues to build upon ongoing research by Kellar *et al.* assessing stress levels in odontocetes associated with Navy sonar exercises at the Southern California Offshore Range (SCORE). Novel laboratory techniques, recently developed at Southwest Fisheries Science Center (Kellar *et al.* 2006, Kellar *et al.* 2009), are successfully being used to measure steroid hormones in



marine mammal blubber indicating that these studies are both realistic and feasible. The combined results of these studies at both AUTECH and SCORE will provide greater power for assessing the extent and magnitude of stress responses in cetaceans exposed to sonar.

## RELATED PROJECTS

### *Monitoring beaked whale movements during the Submarine Commanders Course using satellite telemetry*

This project is a collaborative project between the Bahamas Marine Mammal Research Organisation, NOAA Southwest Fisheries Science Center and the Naval Undersea Warfare Center (David Moretti). Satellite telemetry is being used to monitor the movements and diving behavior of beaked whales and other odontocete cetacean species on the US Navy's Atlantic Undersea Test and Evaluation Center (AUTECH) range before, during and after sonar exercises in which multiple ships are using their tactical sonars. Field work during this project is providing opportunity to collect biopsy samples and photo-identification data at AUTECH. This project has been supported by the US Department of Defense (NACFAC - Living Marine Resources Program).

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## **PUBLICATIONS**

Kellar N, Catelani K, Robbins M, Trego M, Allen C, Danil K, Chivers S (2015) Blubber cortisol: A potential tool for assessing stress response in free-ranging dolphins without effects due to sampling. *PLoS One* 10: e0115257 [published, refereed]

**Appendix 1. Table 1. Model averaged coefficients for factors associated with blubber cortisol, median, and 95% probability interval values. % selected: percent of the iterations that each factor was selected to be included in the final model. Those with positive median values indicate direct relationships and negative values indicate inverse relationships. Highlighted values are those with evidence favoring inclusion in the final models. Tables are in order for Mden, Pmac, Meur.**

Mden Factor	Model coefficients			
	2.50%	median	97.50%	%selected
Area	-0.096	0.093	0.108	0.71%
Sex	-0.266	-0.103	0.080	0.51%
Pregnancy State	-0.241	-0.058	0.119	0.33%
Latitude	-0.130	-0.011	0.129	0.01%
Ordinal date (Year)	-0.746	-0.501	-0.311	99.99%
Julian date	-0.281	-0.051	0.175	0.40%
%Lipid	-0.399	-0.210	-0.090	82.32%

Pmac Factor	Model coefficients			
	2.50%	median	97.50%	%selected
Area	-0.222	0.731	0.103	0.33%
Sex	-0.230	0.014	0.176	0.89%
Pregnancy State	-0.210	-0.034	0.139	0.22%
Latitude	-0.289	-0.060	0.129	0.29%
Ordinal date (Year)	-0.276	-0.057	0.182	0.44%
Julian date	0.111	0.212	0.375	92.56%
%Lipid	-0.201	-0.057	0.175	0.62%

Meur Factor	Model coefficients			
	2.50%	median	97.50%	%selected
Area	-0.159	0.031	0.213	0.99%
Sex	-0.196	-0.093	0.088	0.65%
Pregnancy State	-0.140	-0.058	0.120	0.73%
Latitude	-0.205	-0.060	0.129	0.60%
Ordinal date	-0.203	-0.003	0.198	0.03%
Julian date	-0.121	0.001	0.125	0.04%
%Lipid	-0.281	-0.051	0.175	0.41%